Horticultural Fellowship Awards

Interim Report Form

Project title:	Succession planning to sustain the UK's expertise in field and laboratory plant pathology research and development
Project number:	CP 90
Project leader:	Dr Angela Berrie East Malling Research
Report:	Annual report, March 2015 (Year 4)
Previous report:	Annual report, November, 2014
Fellowship staff: ("Trainees")	Dr Robert Saville
Location of project:	East Malling Research
Industry Representative:	Andrew Tinsley, Horticultural Development Company
Date project commenced:	7th November 2011
Date project completed (or expected completion date):	6th November 2016

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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Progress Against Objectives

Objectives

Objective	Original Completion Date	Actual Completion Date	Revised Completion Date
1. Identify and recruit a successor with the most appropriate background to act as understudy to Dr Berrie.	07/11/11	07/11/11	
2. Develop and deliver a training programme to provide the post-holder with skills and experience in the identification of field and laboratory pathology and an ability to conduct and advise on commercial disease management strategies.	06/11/16	ongoing	
3. Facilitate the development of a successor to Dr Berrie through a programme of collaboration (with other technical experts outside EMR), education, demonstration and shadowing, and industry communication to provide the successor with the skills to deliver practical disease management R&D in fruit and other perennial crops.	06/11/16	ongoing	
4. Enable the post-holder to instigate their own sources of income and the delivery of strategic and applied R&D to act as the means to sustain future innovation within commercial horticulture.	06/11/16	ongoing	

Summary of Progress

1. Identify and recruit a successor with the most appropriate background to act as understudy to Dr Berrie. **Completed**

Robert Saville commenced employment at EMR in November 2011. Robert Saville joined EMR having attained his PhD at the John Innes Centre, Norwich working on the dwarfing genes of cereals, their role in cell development and their pleiotropic effects on disease. The combination of experience working with different pathosystems and molecular techniques provide a good foundation to fulfil the subsequent objectives.

2. Develop and deliver a training programme to provide the post-holder with skills and experience in the identification of field and laboratory pathology and an ability to conduct and advise on commercial disease management strategies. **Ongoing**

The training programme during the reporting period has consisted of a reduction in time spent on specific fellowship projects and an increase in time invested in the procurement and management of new and current research projects undertaken in the pathology group (as detailed in Objective 4).

3. Facilitate the development of a successor to Dr Berrie through a programme of collaboration (with other technical experts outside EMR), education, demonstration and shadowing, and industry communication to provide the successor with the skills to deliver practical disease management R&D in fruit and other perennial crops. **Ongoing.**

During the reporting period interactions with industry and scientific experts have continued, providing valuable knowledge transfer and collaborative opportunities for the future.

Presentations at the SCEPTRE conference, and AHDB days have enabled the communication of research outcomes to the industry. Also attendance at industry events such as Fruit Focus and National Fruit Show have provided opportunities to interact directly with the industry in addition to; regular attendance at EKFS farm walks, AGM and committee meetings along with marketing/agronomy group meetings (inc. TWF, BGG, WWF, Hutchinson's), farm visits e.g. Clive Baxter, William Wollmer and Nick Dunn and hosting industry representatives including Amanda brooks (AG Thames), James Shiltoe (FAST), Ken Jeffery (fruitFed supplies,NZ), Richard Heathcote (NACM), Morgan Rogers (Turner and growers, NZ) and Andrew Barclay (BGG).

Hosted numerous external scientists through role as seminar organiser (inc. Paul Neve, Simon Potts, Amandea Rasmussen, John Crawford, Fergus Lowe and Miriam Gifford). Attended and presented at several conferences/meetings (e.g. Nornex meeting, BSPP conference (14-15

September, Bristol), European canker workshop (12th November, Kent), AAB IPM 10 year plan (18-19th November, Grantham) and Apple lenticel rot workshop, 15-17 December, Paris). Hosted scientists visiting the site including James Woodhall (Fera) and Sean Macantsaoir (AFBI, NI).

4. Enable the post-holder to instigate their own sources of income and the delivery of strategic and applied R&D to act as the means to sustain future innovation within commercial horticulture. **Ongoing**

Lead for a multi-partner consortium project to the AHDB Tree Fruit Panel for the IPM of Tree Fruit Pests and Diseases.

Also manages a portfolio of projects from various funders including AHDB, InnovateUK, BBSRC and industry.

Lead on BBSRC LINK proposal (in preparation). Already secured 25% of funding through AHDB panels.

Lead on two IUK proposals (failed)

AHDB PhD studentship, understanding endophytes of apple (awarded)

Supervises a full time research assistant and summer students (e.g. BSPP summer vacation bursary scheme).

Milestones not being reached

All milestones are being reached.

Do remaining milestones look realistic?

All milestones have a realistic completion date.

Training undertaken

In addition to the on-the-job training, detailed above, formal training within the reporting period is as follows;

PhD supervisor training

In house project costing tool training

Disciplinary and Grievance training

Expertise gained by trainees

In addition to the expertise gained from the activities described above the trainee has added to his publication record through major contributions to the following publications;

Xiangming Xu, Thomas Passey, Feng Wei, **Robert Saville** and Richard J. Harrison (2015) Amplicon-based metagenomics identified candidate organisms in soils that caused yield decline in strawberry. Horticulture Research. 2, 15022; doi:10.1038/hortres.2015.22

Other achievements in the last year not originally in the objectives

Committee member of the East Kent Fruit Society

Institute pesticide officer

Plant health officer

Changes to Project

Are the current objectives still appropriate for the Fellowship?

Fellowship objectives remain unchanged

Grower Summary

The nature of the fellowship projects means that a grower summary is not appropriate at this stage.

Science Section

Objectives

As part of the training fellowship three projects were initially proposed to encompass some of the training requirements vital for field and laboratory plant pathology research and development. As the fellowship has progressed through the objectives. The emphasis has shifted this year from field and laboratory training through project work (objective 2) to training in procurement and management of projects (objective 4). Herein the project work carried out during the fourth year of the fellowship is reported;

(1) Continuation of the apple rot survey and determination of the causative agents of apple rots to contribute towards the sustainable control of storage rots of apple.
(2) Utilising a new metagenomic assay in development at EMR to determine the endophytic profile within commercial strawberry plants for future research on the role of endophytes on plant tolerance/resistance to pests/diseases in relation to host genotypes and external conditions.

Project 1: Sustainable control of storage rots of apple

1.1 Introduction

Fungal rots can result in significant losses in stored apples, particularly in fruit stored beyond January. Certain pack houses will record losses due to rots for individual bins of fruit, thus relating the loss to particular orchards, harvest time and pre-harvest factors, however they rarely identify the rots present. It is important to identify the rot profile in stored apples over time to build a dataset (including orchards, harvest time and pre-harvest factors) from which to base management strategies. In previous surveys *Nectria*, *Botrytis*, brown rot (*Monillinia*), *Penicillium*, *Phytophthora* and *Gloeosporium* have been identified as the main rots in apple. Other rots such as those caused by *Colletotrichum sp., Fusarium sp., Botryosphaeria sp.* and *Phomopsis sp.* have been increasing in incidence. A greater understanding of the epidemiology and orchard factors contributing to rot development has helped in informing management strategies to reduce their prevalence.

The concept of rot risk assessment was introduced via the Apple Best Practice Guide (Webster *et al*. 2001). The rot risk assessment takes account of various pre-harvest factors to predict the level of rot likely to occur in store and thus inform a management strategy, be it pre-harvest treatments, selective picking or storage term, to minimise losses in store. The

factors used for rot risk assessment are; daily rainfall, orchard factors, fungal inoculum (particularly brown rot and canker), crop load, % bare ground (*Phytophthora*), % crop <0.5 metre from the ground, orchard rot history and fruit storage potential (mineral composition and firmness). For example, *Phytophthora* rot risk is influenced by three key factors; Rainfall in the 15 days prior to harvest, % bare ground and % crop <0.5 metre from the ground (Table 1).

Table 1. Factors influencing the risk of *Phytophthora* rot (from Apple Best Practice Guide, Webster et al. 2001)

Factor	Criteria for risk
(1) Rainfall in 15 days prior to harvest	low or no rain = low risk 20 mm or >= high risk
(2) % bare ground	100% bare ground (overall herbicide) = high risk 0% bare ground (overall grass or mulch or weed cover) = low risk
(3) % crop <0.5 metre from theground	15% or >= risk

In addition to rot risk assessment other management strategies can be employed to minimise losses in store such as selective picking whereby only undamaged fruit is harvested and all fruit below 0.5 metres above the ground is excluded. This reduces the risk of introducing fungal rots, such as brown rot and *Penicillium* rot which establish on damaged fruit, and also *Phytophthora* rot which is prevalent on low hanging fruit, into the bin.

Pre-harvest fungicides applied for rot control are generally applied 2-4 weeks before harvest resulting in a high risk of residues in the fruit. By applying the recommendations set out in the rot risk assessment as part of an IPM approach, such treatments could be avoided thus reducing the risk of pesticide residues on fruit whilst reducing the financial and environmental costs of pesticide application.

Data available from rot surveys undertaken over the last 80 years reveal interesting trends in the rot profile over time (Figure 1) which reflects changes in apple growing practices. Fungicide use (chemistry available and application timing) have changed markedly; from post-harvest drenching in the past to flowering and pre-harvest application currently. Advances in storage technologies has led to significant reductions in losses and also influenced the rot profiles observed. Barn stored fruit in the 1930's in which average losses in Cox of over 25% were recorded and the dominant rots were caused by *Neofabraea* sp. and Botrytis whilst modern day refrigerated controlled atmosphere storage with ethylene management technologies to

control ripening have reduced average losses to Cox of less than 3% and the dominant rots are caused by *Monilinia fructigena* and *Neonectria ditissima*. In addition, changes in climatic conditions and orchard practice will influence the rot profile over time.

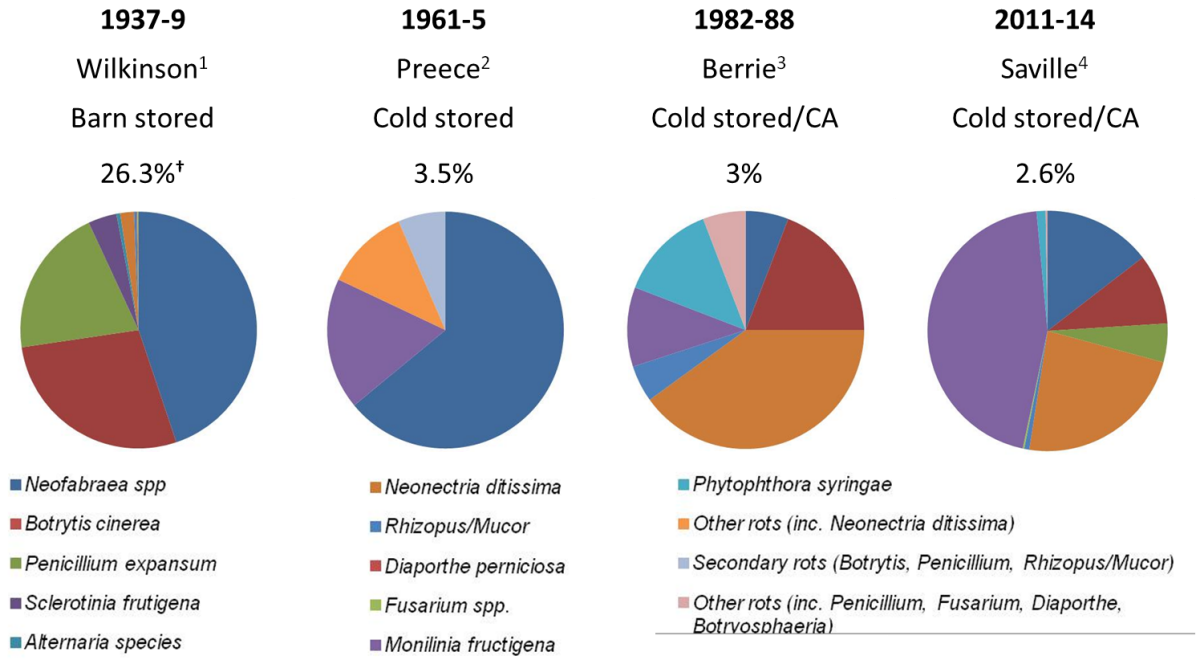

Figure 1. Data compiled from four rot surveys spanning the last 75 years. The data set is for Cox seeing as this is the common cultivar recorded across all surveys. [1]Wilkinson, 1984, [2]Preece, 1967, [3]Berrie, 1989, [4]Saville, 2013, † Average total losses due to rots during the survey period. The categorisation of taxa in the legend are described as recorded in the literature so some inconsistencies between data sets are present i.e. *Sclerotinia frutigena* is a synonym of *Monillinia fructigina* and rots have been grouped in certain surveys e.g. 'other rots'. As far as possible common colour coding has been used to represent these inconsistencies.

The recent trend observed from the rot survey data of increasing incidence in Glosporium rot (caused by *Neofabraea* sp.) lead to a study to investigate the species identification. During the rot surveys conducted from 2013 and 2014 a collection of Neofabraea isolates was assembled and molecular methods were used to identify the distribution of the species present. The results (reported in 2014 interim report) shows that three species of Neofabraea are present in the UK; *N. Alba* (now referred to as *N. vagabunda*), *N. perennans* and *Crytosporiopsis kienholzii*. Samples were taken from Kent and Herefordshire and there was a clear difference in species distribution with *N. alba* being the dominant species in Kent whilst *N. perennans* was dominant in Herefordshire. This study was the first record of *Crytosporiopsis kienholzii* in the UK (manuscript for new disease report in prep).

It is important to continue the rot survey to monitor changes in rot profiles over time and, in turn, inform and prioritise management strategies accordingly. The results from the 2015 rot

survey (reflecting the 2014 growing season) will be presented here in the context of previous rot surveys. In addition a collection of Neofabraea isolates were molecularly characterised to further build on our knowledge of the species distribution of this disease.

1.1 Methods

1.1.1 Survey

Three pack houses were visited in Kent between January and March 2015 (Table 2). Rots were assessed on the grader of whatever variety was being graded at the time of the visit. Rots were identified visually and numbers recorded. Unidentified rots were cultured on to potato dextrose agar and identified from spores or characteristic culture growth.

Table 2. Fruit pack houses visited between January and March 2015

Pack house	**Location**	**Number of times visited**
Newmafruit Farms Ltd	Howfield Farm, Chartham Hatch, Kent	3
F W Mansfield & Sons Ltd	Nickle Farm, Chartham, Kent	3
The Breach	Goudhurst, Kent	6

1.1.2 Molecular identification of the *Neofabraea* species complex

A collection of 20 *Neofabraea* isolates was made during the survey in 2015. Molecular identification was used to determine the species of the isolates. DNA was extracted and amplified from two phylogenetically informative regions (ITS and β-tubulin) and sequenced. The sequence data was then queried against reference sequences of the *Neofabraea* species.

1.2 Results and Discussion

1.2.1 2015 Survey

A total of 24 samples of fruit were surveyed during the 12 visits spanning from mid-January to mid-March. In total 6 different cultivars were surveyed Gala (9), Bramley (4), Braeburn (3), Cox (3), Jazz (3) and Egremont Russet (2). The majority of samples surveyed were picked in September (15) and October (2), a further two were picked in August and the pick date for the remaining sample was unknown. The relatively early picks are indicative of a relatively early growing season experienced in 2014. A summary of the rot survey data is presented in Table 3.

Table 3. Summary table of rot survey data collected during the 2014/15 storage season. The table shows the average percentage loss attributed to each rot for each cultivar recorded during the survey together with the number of samples recorded for each cultivar and the average percentage loss.

	Average % of loss attributed to each rot;															
Cultivar	*Brown rot*	*Botyrtis*	*Phytopthora*	*Penicillium*	*Nectria*	*Gloeosporium*	*Fusarium*	*Mucor*	*Botryosphiria*	*Phomopsis*	Stalk	Eye	Cheek	Core	Number of samples	Loss (%)
Braeburn	11.1	42.9	12.1	14.1	19.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3	1.6
Bramley	54.7	3.0	3.8	10.9	10.9	0.0	9.4	0.0	0.0	0.0	5.1	0.0	1.3	0.8	4	2.5
Cox	21.5	14.1	0.5	7.0	27.3	29.2	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	3	2.2
Egremont Russet	28.4	0.0	0.0	5.0	48.5	18.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2	3.3
Gala	44.8	5.9	0.5	2.2	39.4	6.9	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	9	1.7
Jazz	0.0	39.4	18.2	9.3	23.9	0.0	0.0	3.0	0.0	0.0	0.0	0.0	6.3	0.0	3	0.4
Overall average	**26.8**	**17.5**	**5.8**	**8.1**	**28.3**	**9.0**	**1.6**	**0.6**	**0.0**	**0.0**	**0.8**	**0.0**	**1.3**	**0.1**	-	**2.6**

The rot profile closely corrolates with the weather events of the previous growing season. Consistent with the weather in 2014; a relatively cool, drawn out and at times wet blossom period, all of which are favourable to certain rot causing pathogens. At the other end of the season, another critical period for certain rot causing pathogens, early varieties benefited from a very dry (low risk) harvest period whilst later harvested varieties were exposed to high rainfall (high risk). Accordingly, losses were around average with Bramley experiencing the highest losses (2.5%) and Jazz experiencing the lowest (0.4%) of the cultivars sampled. Brown rot (caused by *Monilinia fructigena*), usually the major rot, was relatively low, which may reflect successful codling moth control during the season, the damage from which is a key entry point for this pathogen. Botrytis, which usually infects during the blossom period and remains latent until storage, was relatively high this year, particularly on Braeburn and Jazz, this may reflect the extended period of blossom which increased the window of infection. Phytophthora, a soil borne pathogen which is favoured by wet weather, was relatively high in late harvested varieties (Braeburn and Jazz picked around mid-October in 2014) whilst earlier varieties escaped the risk thanks to a very dry September (<10mm). Nectria rot (caused by *Neonectria ditissima*) caused equivalent losses to brown rot, particularly in canker susceptible varieties. Gloeosporium (caused by *Neofabraea* species) was present in nearly 30% of samples assessed which is a significant reduction from recent surveys when Gloeosporium was at its peak (almost 80%).

Molecular identification of the *Neofabraea* species complex

During the early part of 2015 whilst conducting the rot survey *Neofabraea* isolates were collected and their species identity determined. Of the 20 isolates collected (from Kent only) 60% were *N. perennans*, 35% were *N. alba* and a single isolate (5%) was *Cryptosporiopsis kienholzii*. This result is in contrast to results reported last year where *N. alba* was dominant in Kent suggesting that the species distribution is very dynamic as from one season to another a huge shift in the dominant species has occurred (Figure 2). This may be related to climatic conditions or may be affected by how early or late the season has been; fruits become more susceptible as they mature and respective species of *Neofabraea* have a period of maximum spore release (also defined by climate), certain varieties or years may escape or coincide with the period of maximum spore release.

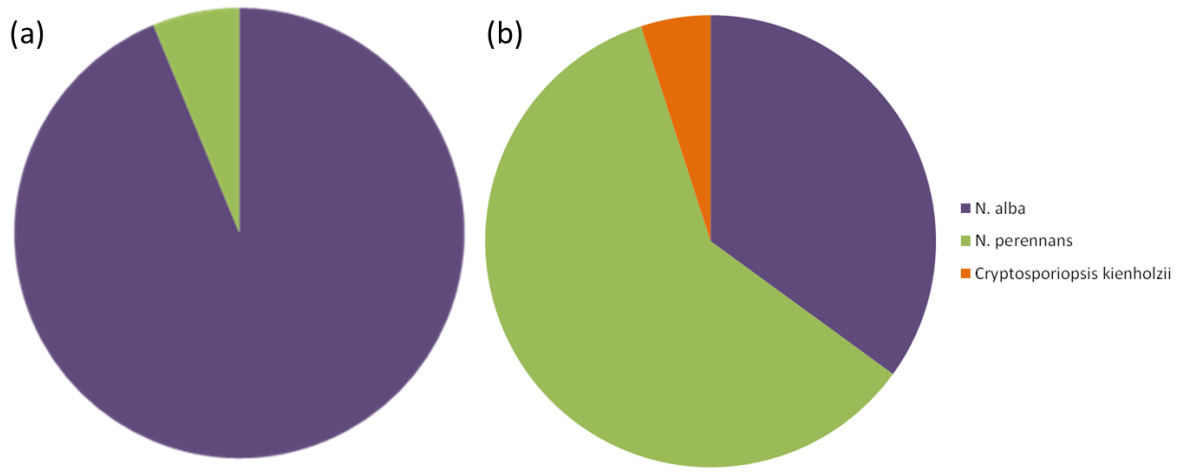

Figure 2. *Neofabraea* species identification of a collection of isolates present during the 2012 and 2013 growing season (a) compared to a collection of isolates present during the 2014 (b) growing season. Both collections were sampled from apples grown in orchards in Kent.

Project 2: Determining the endophytic profile of cultivated strawberry

2.1 Introduction

Endophytes are microorganisms (usually fungi or bacteria) which live within the plant without causing apparent disease. Endophytes are receiving increasing attention internationally as they are recognised as agents which can enhance resistance to biotic attack, enhance abiotic stress tolerance and increase growth due to increased solubilisation of minerals and enhanced nitrogen use efficiency. Although the host-endophyte interaction tends to be mutualistic, other 'shades' of endophytism include commensals, temporary residents, latent pathogens or latent saprophytes.

With the recognition of endophytes as important components to the host, much like the microflora of the human gut is important to health, it is important to know what the profile of these organisms are and what influences their survival.

In the last 10 or so years the field of metagenomics, the study of biological content within environmental samples using molecular techniques, has received increasing attention. The recent advances in DNA based molecular techniques have enabled the characterisation of microbial communities within environmental samples not previously feasible using traditional isolation techniques which required culturing and morphological/biochemical identification. The application of these techniques to various biological questions has uncovered hidden diversity not visible using traditional techniques, not least in the field of endophytic associations with plants.

Using a metagenomics workflow in development at East Malling Research microorganisms living endophitically within cultivated strawberry have been determined. Knowledge of the endophytic profile of strawberry and factors which positively or negatively affect it may have wide implications ranging from pest and disease control, resilience to abiotic stresses and water and fertiliser use efficiency. A potential outcome from this work will be the identification of microorganisms which promote resistance to disease which could be artificially introduced at the pre-planting stage. Latent pathogens (such as *Gnomonia fragariae*) may also be detected in asymptomatic and apparently healthy planting material enabling a better understanding of the epidemiology of strawberry diseases in commercial crops. Further applications could arise with application of this technique to other horticultural crops such as apple.

2.2 Methods

2.2.1 Tissue preparation for the metagenomics workflow

Tissue preparation is an important step to ensure that samples contain true endophytes and are not contaminated with epiphytes living on the surface of the plant tissue. A preliminary experiment was conducted comparing two methods of tissue sterilisation on four tissue types (young leaf, old leaf, petiole and crown (Figure 3)) using strawberry plant material collected from two growing situations (commercial and garden). Reproductive tissues (i.e. flower and fruit) were omitted at this stage due to anticipation of difficulties with DNA extraction.

Figure 3. Tissues types sampled for method optimisation.

In order to kill epiphytes living on the surface of the sampled tissue two published methods of sterilisation were tested;

(i) Chemical sterilisation (modified from Schulz *et al.,* 1993).

Tissue immersed in 100% ethanol for 30 seconds, washed in sterile water, immersed in 33% commercial bleach solution (5% available chlorine) for 5 minutes, immersed in ethanol for a further 30 seconds and then four separate washings in sterile water. All steps were carried out in 25 ml bijous in a sterile flow hood. Tissue samples were dried on sterilised filter paper disks in a sterile flow hood.

(ii) Physical sterilisation (modified from Lundberg *et al*., 2012)

Tissue sections placed in 50 ml falcons with 25 ml of sterile phosphate buffer (7.18 g of NaH2PO4*2H2O + 22.21 g of Na2HPO4*12H2O + 200 µl Silwet L-77 + 1 L RO water). Tissue was sonicated in a Bandelin Sonorex sonicator amended with ice at low frequency for 5 minutes (five 30 second bursts followed by five 30 second rests). Tissue samples were dried on sterilised filter paper disks in a sterile flow hood.

For culturing, split Petri dishes were prepared with water agar (WA; 1.2% w/v, technical agar No. 3, amended with 60 mg L^{-1} penicillin G and 80 mg L^{-1} streptomycin sulphate). To collect tissue for DNA extraction and subsequent metagenomics analysis 2 ml eppendorphs containing 2 ball bearings were prepared.

Three sections of each tissue prepared above was sampled in a sterile flow hood using sterile equipment (scalpel or cork boarer). For leaf material a 6 mm diameter leaf disk was excised, for crown material a 6 mm^2 piece of tissue was excised, for petiole material 6 mm lengths were prepared.

Excised tissue was cut in half. Half of the tissue was pressed onto one half of the split Petri dish (epiphyte press, Figure 4) the same tissue sample was then placed in the centre of the other half of the plate (tissue sample, Figure 4). Samples were incubated at 20°C and monitored.

For each sample the other half of the tissue was placed into the prepared eppendorf (material for each tissue class was pooled) and flash frozen in liquid Nitrogen. These samples were stored at -80 C°.

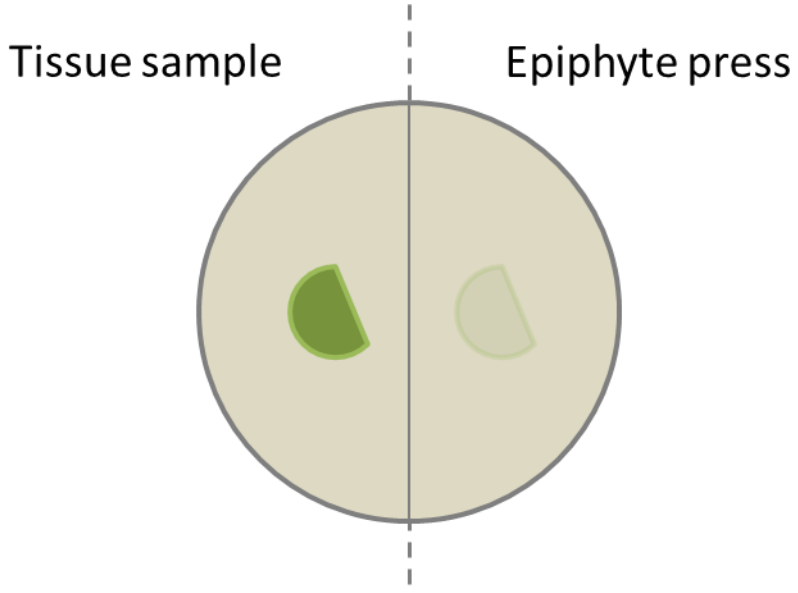

Figure 4. Split Petri dish to determine the effectiveness of the sterilisation techniques.

2.3 Results and Discussion

2.3.1 Optimisation of tissue preparation

Following chemical sterilisation nothing grew on either the epiphyte press or from the tissue sample on any of the tissue samples tested. This suggests that the chemical sterilisation protocol used in this experiment was too severe. The physical sterilisation protocol was not effective. Fungal growth was present on the epiphyte press in addition to the tissue samples for three of the four tissue classes (no fungal growth was observed on the plates with petiole material).

Physical sterilisation was tested because the physical disruption (by vibration) of the surface dwelling communities is thought to (1) kill and (2) destroy DNA of any of the epiphytes present (Lundberg *et al.* 2012). This is important if sensitive molecular approaches such as the metagenomics workflow are used in downstream applications. Chemical sterilisation was tested because this technique has been used extensively for the characterisation of endophytic communities using traditional culturing methods, where residual DNA contamination from epiphytes is not an issue. Other methods tested subsequently included taking epidermal peels of leaves to remove the epiphytic fraction physically but this method, which is only suitable for leaf material, was not considered practical.

2.3.2 Metagenomic analysis

The analysis of the data was conducted using established bioinformatics processes. Unfortunately a large proportion of the reads were for plant DNA for both Prokaryotes (Chloroplast and Mitochondrial DNA) and Eukaryotes (Plant genomic DNA). This has limited the potential of the analysis and will need to be addressed through modifications of the sample preparation protocol in future. The OTUs (operational taxonomic units) which were not derived from plant material are described in Table 4. It should be noted that the bioinformatics process is only as good as the databases it interrogates. The databases used in this study use sequences deposited internationally however this is not all encompassing e.g. the underrepresentation of certain groups e.g. Oomycota (which include *Phytohphthora* and *Phythiums* etc) and an overrepresentation of some ecological groups e.g. free living aquatic microbes which have been catalogued as part of large international metagenomics projects investigating ocean diversity. As a result the closest match is assigned to the OTU's which is not always reflecting actual identity.

Overall 11 prokaryotic phylum and 10 eukaryotic classes (a lower taxanomic rank to phylum) were identified following the (bioinformatics) exclusion of plant DNA (Table 4). Of the prokaryotic phyla represented many were soil dwelling and one, Firmicutes, includes the

Table 4. Taxa for prokaryotes (to phylum level) and eukaryotes (to class level) of the OTUs and their broad ecological niche based on internet searches.

	Taxa	Broad ecological niche
Prokaryotes	Actinobacteria	**Free living** - recognised primarily as soil bacteria
	Armatimonadetes	**Endophyte** - aerobic chemoheterotrophic bacterium, isolated from *Phragmites australis*
	Bacteroidetes	**Free living** - Widely distributed in the environment (soil, sediments, and sea water)
	Chlamydiae	**Pathogen** - obligate intracellular pathogens (not plant)
	Chloroflexi	**Free living** - Aerobic thermophiles
	Firmicutes	**Endophyte/Epiphyte** - Includes *Bacillus subtillus*
	Gemmatimonadetes	**Free living** - recognised primarily as soil bacteria
	Nitrospira	**Free living** - soil bacteria and part of a nitrification process
	Planctomycetes	**Free living** - aquatic bacteria
	Proteobacteria	**Free-living** - responsible for nitrogen fixation
	Verrucomicrobia	**Free living** - Widely distributed in the environment (soil, sediments, and sea water)
Eukaryotes	Chaetomium	**Saprophyte** - found in/on dung or excretions of dung of Herbivores
	Cladosporium	**Saprophyte** - commonly found on living and dead plant material can be pathogenic e.g. on tomato
	Clonostachys	**Endophyte** - also known as Gliocladium (strains have been commercialised as a biological control agent)
	Cryptococcus	**Free living** - yeast living primarily in soil
	Engyodontium	**Saprophyte** - common in soil and plant debris
	Exobasidium	**Pathogen** - of *Ericaceae* (heather/yew) and *vaccinium.*
	Gnomoniopsis	**Pathogen** - of *Fagaceae, Onagraceae* and *Rosaceae.*
	Hymenoscyphus	**Pathogen and Saprophyte** - of *Fraxinus*
	Itersonilia	**Pathogen** - of Chrysanthemum, Gerbera and Parsnip.
	Penicillium	**Pathogen and Saprophyte** - opportunistic
	Trichoderma	**Endophyte** - of several plant species (strains have been commercialised as a biological control agent)

Bacillus genus; well-known and exploited for their biological control properties e.g. Serenade®. Of the Eukaryotes, which had better resolution down the taxanomic ranks, a range of different ecological lifestyles were represented. From the general saprophytes (organisms which live on dead or decaying organic matter) lying in wait for the host to die (e.g. Cladosporium and Penicillium), to the well-recognised beneficial endophytes (e.g. Trichoderma and Gliocladium) and the potential latent pathogens waiting for an opportunity to attack the host (e.g. Gnomoniopsis).

To look at overall effects of treatment we can use statistical methods such as principle component analysis which show overall effects of the OTU profile rather than individual species. This is a useful analysis to see a bigger picture before probing the dataset further to establish the OTU's responsible for the differences. This analysis was done for prokaryote (Figure 5) and eukaryote (data not shown) data sets and show that neither method of sample preparation (chemical sterilisation, physical sterilisation or both) or tissue type/age (Crown and old or young leaves) had a marked effect on the OTU profile observed.

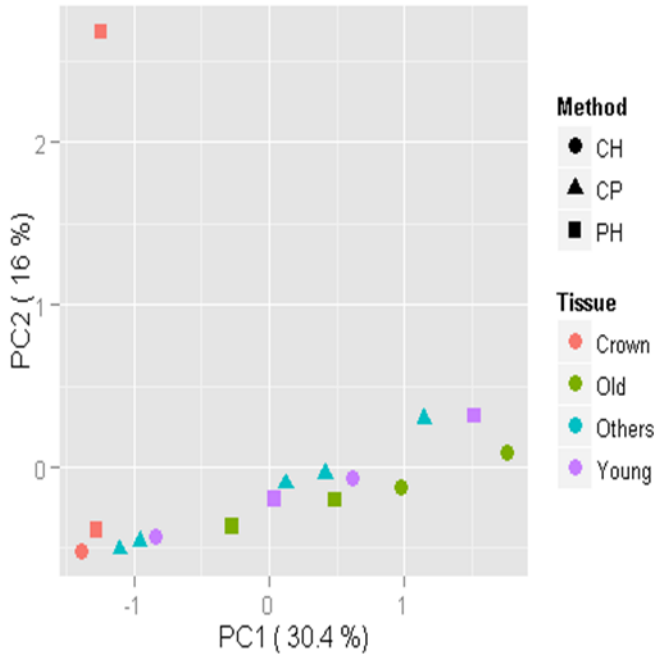

Figure 5. A principle component analysis of the overall effect of different treatments on the OTUs (taxonomic groups) of prokaryotes. Treatments (on right hand side of the graph). Sterilisation method distinguished by shape; CH: chemical sterilisation; CP: Chemical and physical sterilisation; PH: physical sterilisation. Tissue distinguished by colour; Crown: Crown tissue; Old: old leaf; Others: leaves from tissue culture plants; Young: young leaf.

Following from this first experiment a number of modifications to the pipeline have been undertaken. For instance in the first experiment the usable information was diluted by a majority of reads from plant DNA and so our modified tissue preparation protocol now involves

gentle abrasion of the plant tissue with glass beads to liberate microorganisms within rather than complete tissue disruption thus enriching the endophytic fraction relative to the plant DNA. The bioinformatics work flow now interrogates a larger database which include Oomycota. This revised tool is already being applied to elucidate the endophytic profile of apple with particular reference to *Neonectria dittisima* and further work will be carried out in an AHDB studentship to commence in October 2016.

Knowledge and Technology Transfer

Presentation of fellowship project results have been made at various AHDB events, industry events (e.g. BIFGA day) and scientific forums (e.g. European lenticel rot workshop) and HDC/EMT/HTA funding has been acknowledged accordingly. The findings have been featured in AHBD tree fruit and soft fruit review publications and a feature on endophytes will be published in the AHDB grower magazine for cross sector coverage.

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